Acute kidney injury to chronic kidney disease transition: insufficient cellular stress response

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\textbf{Purpose of review} Recent epidemiological and preclinical mechanistic studies provide strong evidence that acute kidney injury (AKI) and chronic kidney disease (CKD) form an interconnected syndrome. Injured kidneys undergo a coordinated reparative process with an engagement of multiple cell types after injury; however, maladaptation to the injury subjects kidneys to a vicious cycle of fibrogenesis and nephron loss. In this review, we will outline and discuss the pathogenesis of AKI-to-CKD transition with an emphasis on dysregulated ‘cellular stress adaptation’ as a potential therapeutic target.

\textbf{Recent findings} Recent studies identify the crucial role of injured tubular epithelial cells in the transition from AKI to CKD. Damaged tubular cells undergo reactivation of developmental and epithelial–mesenchymal transition signaling, metabolic alteration, and cell-cycle arrest, thereby driving inflammation and fibrogenesis. Recent work highlights that cellular stress-adaptive pathways against hypoxic and oxidative stress provide insufficient protection after severe AKI episode.

\textbf{Summary} Insufficient cellular stress adaptation may underpin the persistent activation of inflammatory and fibrogenic signaling in damaged kidneys. We propose that harnessing cellular stress-adaptive responses will be a promising therapeutic strategy to halt or even reverse the deleterious process of AKI-to-CKD transition.

\textbf{Keywords} acute kidney injury to chronic kidney disease transition, Keap1-Nrf2 pathway, stress adaptation

\section*{INTRODUCTION}
Recent epidemiological studies provide fundamental insights into the critical connections between acute kidney injury (AKI) and chronic kidney disease (CKD) \cite{1,2}. Previously, acute and chronic renal failure were considered two distinct syndromes because of the long-held belief that the tremendous regenerative capacity of the kidney was sufficient to reverse acute renal failure. However, the complete recovery of AKI is less common than previously assumed and the field now recognizes that AKI and CKD form an interconnected syndrome. AKI is a major risk factor for the development of CKD and end-stage renal disease (ESRD). AKI episodes accelerate the progression of CKD whereas preexisting CKD predisposes patients to AKI \cite{1,3}. Moreover, AKI and CKD both increase the risk of cardiovascular adverse events \cite{1,4}. Therefore, it is crucial to elucidate the molecular basis of this deleterious link to identify new therapies. In this review, we will discuss the pathogenesis of AKI-to-CKD transition with an emphasis on new findings in recent publications and a focus on the dysregulated cellular stress adaptation as a potential target for this pathological process. We will also propose that reactivation of nuclear factor erythroid 2-related factor 2 (Nrf2), a central transcriptional regulator of antioxidative cellular defense, is a promising therapeutic strategy for this devastating process.

\section*{OVERVIEW OF ACUTE KIDNEY INJURY TO CHRONIC KIDNEY DISEASE TRANSITION}
Experimental data accumulated in past decades led to our understanding that development of CKD after AKI could be because of a maladaptive cellular
response or misdirected repair [5–9] (Fig. 1). Upon injury, kidneys undergo repair processes, such as dedifferentiation, stress adaptation, metabolic alteration, inflammatory cell infiltration, extracellular matrix production, and hypertrophy of remnant nephrons. These processes are mutually reinforcing and require the coordination of multiple cell types in the injured kidneys. When successful, these orchestrated reparative processes allow damaged kidneys to establish a new steady state homeostasis and recover from the injury (a process known as adaptive repair). However, the same processes and signaling cascades can be misdirected or maladaptive when they are either excessive, insufficient, sustained, or mistargeted, resulting in malfunction and scarring of the kidney (known as maladaptive repair) [10]. Pathologic features of maladaptive repair include, but are not limited to, tubular atrophy, excessive and persistent inflammation, extracellular matrix deposition, vascular rarefaction, and reduced nephron mass. Clinical and experimental data indicate that severe and/or repetitive injury overwhelms the regenerative/reparative response and causes it to become maladaptive and misdirected, resulting in progressive decline of renal function [11–13].

Among the cells in the kidney, renal tubular epithelial cells, especially damaged/activated proximal tubular cells, represent a major driver of maladaptive processes [8,10,11]. Selective genetic damage to tubular cells initiates the development of pathological features of CKD [11,13]. Multiple molecular mechanisms have been identified in which damaged tubular cells drive fibrogenesis. Damaged tubular epithelial cells characteristically express kidney injury molecule 1 (Kim1, also known as Tim1), which acts as a receptor for engulfing debris inside the tubular lumen and protects from ischemic AKI [14–16]. However, sustained tubular overexpression of Kim1 is implicated in CKD pathogenesis, indicating that successful recovery requires initial expression and then subsequent downregulation of Kim1 [17]. Damaged tubular cells undergo dedifferentiation, cell cycle alteration [18], reactivation of developmental gene programs such as Sox9, Wnt/β-catenin, Notch, and Hedgehog/Gli signaling.
pathways [19*,20,21,22*,23,24,25*,26*,27,28], and epithelial–mesenchymal transition (EMT) gene program [29,30]. Persistent activation of these programs and cell-cycle alteration drives renal fibrosis [29–31].

Therapeutic strategies targeting the AKI-to-CKD transition can be divided into two major categories: reducing the severity of AKI insult or directing the regenerative process towards beneficial adaptive repair pathways. However, although the targets selected for each of these targeting strategies are distinct, their effect on renal health outcomes could be synergistic as severe and repetitive damage in AKI is the primary trigger of the misdirected cellular response that causes failed recovery in mice and humans [1,11,13]. Recent studies have identified many molecular targets and cellular events that contribute to the maladaptation; the roles of pericytes in peritubular capillary rarefaction and fibrosis [23,32*,33,34], dysregulated vascular growth factor signaling [35**,36–38], enhanced luminal clearance of dead cell debris [39**], DNA damage response [40], uncontrolled inflammation [41,42,43–46], new insights into TGFβ signal regulation [31,47**], epigenetic alterations [48,49,50**], mitochondrial biogenesis and metabolic alterations [27,28,51–53,54**,55,56*,57,58], protective neural circuits to control renal inflammation [59,60**], critical connections of fibrosis and renal anemia [32*,61–65], targetable regulated cell death pathways [66–71], and more. Emerging new technologies also will aid in advancing these therapeutic strategies [72**,73**].

Hypoxic and oxidative stresses [excessive accumulation of reactive oxygen species (ROS)] have been widely considered central aggravating factors of kidney diseases including AKI and CKD [74–76]. Cells under these stressors activate adaptive gene programs driven by master transcription factors including hypoxia-inducible transcription factor (HIF) for hypoxia and Nrf2 for oxidative stress. Interestingly, accumulating evidence suggests that these adaptive pathways appear to provide insufficient protection in mouse models of tubulointerstitial injuries such as unilateral ureteral obstruction (UUO) and ischemia–reperfusion injury (IRI) [32*,77*]. UUO reduced the protein abundance of HIF in chronic anemic kidneys and down-regulated the multiple essential HIF target gene expressions, suggesting that hypoxia-sensing and HIF transcriptional activity are malfunctioning in the injured organ [32*]. Genetic activation of HIF has dual roles in kidney disease models and can be either protective or deleterious depending on when, where, and how the signal is activated [78–80]. Pharmacological activation of HIF has shown protective effects on tubular cell damages and inflammation in unilateral IRI model, which are dependent on endothelial expression of HIF2α [79]. Similarly, Nrf2 activation, which governs cellular antioxidative adaptation, is insufficient and transient in AKI and CKD mouse models whereas the oxidative stress is a major pathogenic driver of disease progression [75,76,77*,81]. Postischemic pharmacological activation of Nrf2 has therapeutic benefits. Currently, multiple clinical trials targeting these cellular stress adaptive pathways are underway [82,83]. In the following, we will summarize recent advances of the Nrf2 pathway and discuss the potential to prevent or halt the maladaptive repair mechanisms by boosting the Nrf2 activity.

KEAP1–NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 ANTI-OXIDATIVE PATHWAY: MASTER REGULATOR OF REDOX HOMEOSTASIS

Nrf2 maintains cellular redox balance and is a master regulatory transcription factor of the cellular antioxidative response. This is accomplished through transcriptional activation of its target genes, which include members of redox regulatory pathways such as glutathione synthesis and recycling, and NAD(P)H synthesis, a critical reducing equivalent for cells [84–89]. Nrf2 also regulates the genes for scavenger receptors, xenobiotic metabolism, mitochondrial biogenesis and motility, iron levels, inflammation, and more [90*,91–93]. Intensive proteomics and transcriptomic analyses have recently identified Nrf2-regulated pathways in mouse kidneys using an Nrf2 inducer, 2-cyano-3,12-dioxyoolean-1,9-dien-28-oic acid (CDDO) methyl (also known as bardoxolone methyl) [94]. Nrf2 has been extensively investigated in preclinical and clinical studies as a promising disease-modifying target against multiple diseases, including kidney diseases [75,76,95]. Outside the field of nephrology, the Nrf2-activating drug BG-12 (dimethyl fumarate, marketed as Tecfidera) is currently widely used in treating multiple sclerosis.

Nrf2 activity is mainly regulated by its abundance within the cell, which is controlled through both Kelch-like ECH-associated protein1 (Keap1)-dependent and Keap1-independent regulatory mechanisms (Fig. 2). Keap1 is an adaptor protein of the Cullin 3-based E3 ubiquitin ligase and directly interacts with Nrf2, promoting ubiquitination of Nrf2. Ubiquitinated Nrf2 is rapidly degraded by the proteasome. Under oxidative stress conditions, key reactive cysteines in Keap1 undergo oxidative modification, disrupting the Keap1–Nrf2 interaction and stabilizing the transcription factor. Disruption of Keap1-mediated Nrf2 degradation results in rapid accumulation of Nrf2 in the nucleus and activation of a gene expression program to restore
redox homeostasis [96,97]. Another mechanism to control the Keap1 activity has been discovered. p62/SQSTM1 is an adaptor protein and substrate for selective autophagy, which accumulates in cells with autophagic dysfunction [98,99]. p62 interacts with and sequesters Keap1 in an autophagosome, thus liberating Nrf2 from Keap1-mediated degradation. The implications of p62-mediated regulatory mechanisms in kidney disease are still not clear.

In addition to Keap1-mediated degradation, Nrf2 abundance is regulated by Keap1-independent pathways, which provide additional drug targets that might be used to activate Nrf2-mediated cytoprotective pathways. One of these Keap1-independent degradation pathways is glycogen synthase kinase 3 (GSK3)/β-transduction repeat-containing protein (β-TrCP)-dependent Nrf2 degradation [100]. GSK3 phosphorylates Nrf2 and phospho-Nrf2 is recognized by the ubiquitin ligase adapter protein β-TrCP and proteosomally degraded. The inhibition of GSK3-β induces Nrf2 in podocytes and promoted therapeutic effects in mouse models of FSGS [101*].

Recently, a second mechanism of Keap1-independent Nrf2 regulation was identified when Donna Zhang and colleagues described the role of endoplasmic reticulum (ER)-stress in Nrf2 regulation. ER-stress induces Hrd1 (HMG-CoA reductase degradation protein 1), an ER-transmembrane E3 ubiquitin ligase, in

**FIGURE 2.** Regulatory mechanism of the nuclear factor erythroid 2-related factor 2 pathway. Nrf2 signal activity is mainly regulated at the level of its protein abundance by the proteasome. (a) ‘Keap1 cysteine-mediated regulation’ is the canonical pathway. Oxidative stress modifies key cysteine residues of Keap1, impairing ubiquitination of Nrf2 and its degradation. (b) In autophagic dysfunction (e.g., observed in hepatocellular carcinoma), accumulated and phosphorylated p62 binds to Keap1 and impairs Nrf2 degradation. (c) GSK-3 mediated phosphorylation of Nrf2 leads to its degradation through β-TrCP-dependent manner. (d) ER-stress induces Hrd1 (E3 ubiquitin ligase) expression and enhances degradation of Nrf2 in certain disease conditions such as cirrhosis. Ub, ubiquitin; Cys, cysteine; ER, endoplasmic reticulum; Nrf2, nuclear factor erythroid 2-related factor 2.
mouse and human fibrotic livers where Nrf2 activity is compromised. Hrd1 enhances degradation of Nrf2 through its ubiquitination, thus explaining the impaired Nrf2 response in cirrhosis [102,103].

The Nrf2 pathway also has important implications in cancer biology. Multiple human cancers have been identified with Nrf2-activated status. The Nrf2-activated-cancers arise through various mechanisms including somatic mutations of Keap1–Nrf2, epigenetic silencing of Keap1, cancer metabolite-mediated Keap1 inactivation, p62-mediated Nrf2 induction, and more [104]. Preclinical models identify that high Nrf2 activity in cancer cells confers survival benefits and chemoresistance. Currently, investigators in the field of oncology are seeking Nrf2 inhibitors as a new class of cancer therapy. These mechanisms developed by cancer, which activate Nrf2 signaling may provide the path to activate Nrf2 pharmacologically and could also be utilized to treat kidney diseases.

**KEAP1–NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 PATHWAY: NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 IN ACUTR KIDNEY INJURY MODELS**

Oxidative stress is a key driver of multiple kidney diseases. Numerous investigators have tested the role of Nrf2 in a preclinical kidney disease model. The initial studies led by Rabl highlighted the protective function of Nrf2 against toxic and ischemic AKI [105,106]. Nrf2 knockout mice showed more severe damages and higher mortality in these AKI models [106]. Conversely, renal damage was reduced by treatment with CDDO-imidazole, a Keap1 inhibitor, which increases Nrf2 activity [105]. The group identified a beneficial immunomodulatory function of Nrf2 in AKI, as T-cell-specific genetic activation of Nrf2 alleviated the AKI severity [107] (Fig. 3).

Potential beneficial effects of Nrf2 activation were identified in long-term renal outcomes and development of CKD diseases after ischemic AKI [77*,81]. In a mouse model of unilateral IRI, initial histological tubular damage is confined to the outer medulla and medullary ray. Subsequently, extensive cortical remodeling, cortical tubular loss, and matrix deposition are observed. In our studies, genetic and pharmacological Nrf2 activation reduced the cortical remodeling and tubular atrophy. The delayed administration of Nrf2 activator showed comparative protection as preemptive treatment, suggesting that Nrf2 activation may have benefits both in reducing the severity of AKI and directing the regenerative process towards beneficial adaptive repair pathways. Conversely, Nrf2 deficiency aggravated cortical tubular loss and resulted in nonfunctioning atrophic kidneys. Notably, despite the progressive tubular loss and persistent oxidative damages, the Nrf2-regulated gene program is only transiently activated (less than 24 h) and not sustained. This finding suggests that Nrf2 activity was repressed by unknown mechanisms. We surmise that the impaired Nrf2-mediated cytoprotection is a major driver of AKI-to-CKD transition [77*].

Other investigators have tested the role of Nrf2 in a UUO model and found that genetic Nrf2 activation attenuated renal fibrosis similar to the unilateral-IRI model [81]. However, conflicting results are reported in Nrf2 knockout mice in two recent publications [108,109]. One report showed that Nrf2 deficiency worsens progressive tubular damage and fibrosis [109] but surprisingly, another report shows that Nrf2-deficiency reduces fibrosis and inflammation [108]. Bone marrow-derived cells from Nrf2 knockout mice appear to be responsible for these protective effects. The authors suggested that the protective effects of Nrf2 deficiency could be attributed to reduced activation of the NLRP3 (nucleotide-binding domain and leucine-rich repeat containing protein 3) inflammasome in bone marrow-derived macrophages. Further close investigation will be required to fully elucidate Nrf2 function in immune cell functions.

Although the full mechanisms underlying insufficient Nrf2 activation in AKI and AKI-to-CKD transition remain unclear, some clues have been identified. A recent report indicated that intact NADPH oxidase type 4 (NOX4) is required for Nrf2 activation after IRI [110]. Clinical and rodent models have shown that aging is a significant risk factor for AKI and AKI-to-CKD transition. In a premature aging mouse model, Nrf2 is mislocalized and trapped in the subnuclear component, leading to reduced Nrf2 activity and increased oxidative stress [111*]. Although the model used represents a genetic disorder of premature aging, it is plausible that reduced Nrf2 activity in aging may increase the susceptibility of aged kidneys to less severe injuries [112]. It is also possible that non-Keap1-mediated Nrf2 degradation impedes Nrf2 activation. Identifying underlying mechanisms that limit Nrf2 activity may lead to newer therapeutic strategies to treat AKI-to-CKD transition.

**KEAP1–NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 PATHWAY: IS HIGH NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 ACTIVITY ALWAYS PROTECTIVE?**

Recently, ROS has been rediscovered as an essential signaling molecule that is required for regulation of several biological pathways such as hypoxia sensing, cellular proliferation, differentiation, and metabolic
Although excess ROS elicits deleterious effects, eliminating ROS could be detrimental and maintaining appropriate ROS levels might be vital for cells to function in certain disease conditions. We have recently identified that Nrf2 inactivation, which increases ROS accumulation, has survival benefits in a mouse model of preeclampsia [114]. Genetic and pharmacological Nrf2 activation eliminates ROS and results in worsening of maternal and fetal outcomes. It appears that ROS accumulation in preeclamptic placentae is required to promote compensational placental angiogenesis in the model [114].

Developmental deleterious effects of genetic Nrf2 activation in kidneys have been identified [115,116]. Embryonic deletion of Keap1 in renal tubular cells results in hydropnephrosis and reduced water-concentrating ability, potentially because of developmental defects of water reabsorption machinery [115,116]. In contrast, Keap1 deletion in adult renal tubular epithelium did not result in morphological defects and provided protection against AKI through Nrf2 activation, providing further evidence that Keap1 inhibition would be an attractive and well tolerated therapeutic strategy in adult kidneys [77]. Understanding the context-dependent Nrf2 functions in various disease models and in different cell types will be necessary to rationally translate this pathway to the clinic.
KEAP1–NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 PATHWAYS: NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 ACTIVATOR IN CLINICAL TRIALS

In the field of nephrology, several potent Nrf2 activators are currently being tested in clinical trials. Bardoxolone methyl (CDDO-methyl) is an inhibitor of Keap1 and Iκ kinase B (IKKβ, NFKB regulator), which activates Nrf2 and suppresses NFKB [117]. CDDO-methyl was tested in diabetic kidney disease (DKD) and increased estimated glomerular filtration rate (eGFR) in a phase 2 clinical trial (BEAM study) [118]. However, a subsequent phase 3 clinical trial with advanced diabetic kidney disease was prematurely terminated because of increased hospitalization related to heart failure, probably caused by fluid overload in CDDO-methyl arm (BEACON study) [119]. To minimize the potential fluid overload, the drug was tested in DKD patients with lower heart failure risk in the TSUBAKI study (NCT02316821) [120]. The study showed safe increase of insulin clearance without cardiovascular adverse effects. The drug also safely increased eGFR in patients with Alport syndrome (CARDINAL, phase 2; NCT03019185) and currently a phase 3 trial is underway [121]. With the promising recent data, the PHOENIX trial (phase 2; NCT03366337) is currently ongoing in testing CDDO-methyl for autosomal dominant polycystic kidney disease, IgA nephropathy, CKD associated with type 1 diabetes, and focal segmental glomerulosclerosis (FSGS). An electrophilic nitrofatty acid (CXA-10, 10-nitrooleic acid) has been shown to potently activate Nrf2 and inhibit NFkB. This drug has protective effects in preclinical models of kidney diseases [122], and will be tested for FSGS in a phase 2 clinical trial.

CONCLUSION

Understanding how and why renal reparative response falls into maladaptation is an intensive area of research. Harnessing cellular stress responses, such as the Nrf2 antioxidative stress pathway, will be a promising therapeutic strategy to halt or even reverse the deleterious process of AKI-to-CKD transition. Further studies will be required to elucidate the molecular underpinnings of the beneficial effects of Nrf2 function and impaired Nrf2 activity in this debilitating disease process.

Acknowledgements

Authors thank Drs Tsuyoshi Inoue (University of Virginia) and Benjamin R. Thomson (Northwestern University) for critical review of our early manuscript.

Financial support and sponsorship

This study was supported in part by a start-up grant from Duke University for T.S., and a Next Generation Leading Research Fund for 2017 of Kagawa University Research Promotion Program for D.N.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

16. This article and the article by Kumar et al. [20] report that the transcription factor Sox9 reemerges in injured tubular cells. The Sox9 is functionally required for renal repair.
Renal pathophysiology


This article reports selective overexpression of Wnt1 from proximal tubular cells sufficiently induce renal fibrosis, confirming the model that paracrine soluble mediator mediates kidney fibrosis.


Kramann reports that persistent activation of Wnt/beta-catenin signaling is a determinant of DMD-to-CKD transition, whereas the transient activation of this signal is suggested to be beneficial.


This article reports that fibroblast/pericyte-selective deletion of beta-catenin attenuated AKI-induced injury in contrast to the aggravating effects of tubular specific deletion of beta-catenin (Gho et al. Kidney Int. 2012).


This study identifies an epigenetic mechanism how fibrotic kidneys lose erythropoietin (Epo) expression. Resolution of TGFβ1-induced DNA hypermethylation in Epo promoter Epo expression in fibrotic kidneys.


This study reports that autocrine loop of TGFβ1−IL11 in fibroblasts drive tissue fibrosis in heart and kidneys.


This article identifies an epigenetic mechanism how fibrotic kidneys lose erythropoietin (Epo) expression. Resolution of TGFβ1-induced DNA hypermethylation in Epo promoter Epo expression in fibrotic kidneys.


This article reports the detailed characterization of mitochondrial disease and alteration of glycolytic enzymes in proximal tubules after IR-induced AKI.


This study identifies an epigenetic mechanism how fibrotic kidneys lose erythropoietin (Epo) expression. Resolution of TGFβ1-induced DNA hypermethylation in Epo promoter Epo expression in fibrotic kidneys.


73. This article reports a nanomedicine approach, fibroblast carbonic anhydrase II, that enables two siRNAs to the renal proximal tubular cells to treat AKI.


75. This article reports a new method to activate endogenous expression of gene-of-interest using CRISPR-Cas9 mediated epigenetic modulation. Using this method, the authors successfully treat a mouse model of AKI.


79. This article shows the potential protective effects of postsischemic Nrf2 activation for AKI-to-CKD transition in a mouse model of unilateral ischemia reperfusion injury.


88. This article reports a new method to activate endogenous expression of gene-of-interest using CRISPR-Cas9 mediated epigenetic modulation. Using this method, the authors successfully treat a mouse model of AKI.


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